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A STUDY OF THE BIOLOGICAL ESSENCE OF THE VIRULENT PROPERTIES OF MICROB

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A STUDY OF THE BIOLOGICAL ESSENCE OF THE VIRULENT PROPERTIES OF MICROBES

Report II

The Nature of Pneumococcus Infection in White Mice Depending on the Route of Infection and the Dose of Causative Agent *

/Following is the translation of an article by S. A. Anatoliy, Institute of Experimental Medicine, USSR Academy of Medical Sciences, Leningrad, published in the Russian-language periodical Zhurnal Mikrobiologii Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), No 2, 1965, pages 120-124. It was submitted on 18 Jul 1963. Translation performed by Sp/7 Charles T. Ostertag Jr./

* Report I. Concerning Methods for a Comparative Study of Virulent and Avirulent Pneumococcus Cultures. Yearbook of the Institute of Experimental Medicine, USSR Academy of Medical Sciences, Leningrad, 1960.

The dependency of the development of infection on the route of infection of the macroorganism has been studied by many authors in respect to various causative agents (Manenkov, 1929; Strukov, 1948; Dvinyaninov, 1950; Ostashevskiy, Obratsov, 1961; Kolle and Hetsch, 1929; Stillman, 1923, and others). However, in the literature there are no exhaustive reports concerning the role of the quantitative factor and the routes of entry of the causative agent into the organism in respect to pneumococcus. It is known only that the most effective route for infecting mice with pneumococci are intraperitoneal injections. In this case it is sufficient to introduce only several (sometimes less than 10) microbial cells in order to develop serious sepsis leading to the death of the animal. Infection through respiratory routes does not lead to the development of an infectious process.

The mission of the present report was to clear up the following:
1) the degree of manifestation of the pneumococcus infectious process when the animals are infected by various routes and with various doses; 2) the constancy of death of infected mice with beginning pneumococcus bacteremia; 3) the reaction of the macroorganism following contact with a pneumococcus culture in those cases when contagion does not lead to the formation of an expressed infectious process.

For infecting the animals we used a culture of pneumococcus type II which was maintained by passaging in mice. During the intraperitoneal and intravenous infection the animals received 0.5 ml each of a 24 hour culture in meat-peptone broth containing 5% horse serum. Infection in the palm of

the front extremity was carried out by the method described by Berman and Slavskaya (1955). For this 0.02 ml of diluted culture was administered in the palm. Before intranasal infection the mice were stunned with ether, after which 0.02 ml of the culture was introduced. The dose of live microbes administered to the mice was determined by the quantitative inoculation of the culture on blood agar.

For the purpose of investigating the infected animals seedings were made from the spleen and from the blood. The spleen was ground with 1 ml of a physiological solution and 0.1 ml of the diluted suspension was inoculated in dishes with blood agar. Then the number of microbes in the organ was calculated in the dishes. The blood for seeding was taken from the heart of a destroyed mouse or from an incision on the tip of the tail (when determining the dynamics of bacteremia in the same animals). A drop of blood (about 0.05 ml) was diluted in 1 ml of physiological solution; the seedings were made from several successive dilutions. By this method the number of live microbes in 1 ml of blood was established.

The value of the LD_{50} was calculated according to the method of Reed and Muench (1938). The death of the animals following intraperitoneal infection was allowed for in the course of three days, and for intravenous and intranasal and administration of the culture into the extremity -- in the course of 7--10 days.

In series I of the tests we compared the magnitude of the lethal doses of pneumococcus during various methods of infection. For this four groups of mice (9-11 animals in each) received a suspension of microbes from successive tenfold dilutions, and then the magnitude of the LD_{50} was determined for each group. It turned out that the LD_{50} following the administration of pneumococci into the peritoneal cavity comprised 10--50 microbes, following infection in the palm of the forward extremity -- 4,000 microbes, following intranasal infection it approximated 30 million live microbes. Thus these facts testified that mice are most sensitive to intraperitoneal infection with pneumococcus.

The mice which were infected in the extremity were destroyed in various periods following the injection and seedings were made from their spleen. In 72 and 96 hours following infection in several seedings the growth of colonies of pneumococcus was noted, but in later periods pneumococci were absent in the spleen. With the aim of defining the data obtained we followed the dynamics of bacteremia in the same animals. Blood was taken from the ends of the mice's tails in various periods following infection, and by means of quantitative seedings a determination was made of the content of microbes in it. The mice (groups of 10--15 animals) were infected with three doses of a broth culture: 45,000 - 4,500 - and 450 live microbes per animal. In the mice of the first group substantial bacteremia was observed from the 1--2nd day, and all the animals died on the 3--4th day following infection. Out of 15 mice in the second group 8 died in the period up to 5 days. The death of the animals, as in the previous group, was preceded by expressed bacteremia (from 3 million up to

75 million microbes in 1 ml of blood). There was no growth in the seedings from the blood of 5 of the surviving mice, and in 2 of them on the 2--3rd day following infection 2000--4000 pneumococci per 1 ml of blood were seeded for each one time. Finally, out of the 9 mice which were infected with 450 cells of pneumococci, one died and from 7 pneumococci were seeded out one time (mostly on the third day) in small quantities (200--9400 microbes in 1 ml of blood). Subsequently pneumococci were not detected in the blood of these mice.

An effect similar to that described of the weakly expressed and rapidly passing pneumococcus bacteremia we observed during the administration of small doses of penicillin (2000 units twice in 24 hours) to mice in 30 hours following intraperitoneal infection with various doses of virulent pneumococci (from 10 up to 1050 cells per mouse). Penicillin therapy lasted for 5 days. Blood from the mice was seeded for the course of 7 days, after which observations for the death of the animals lasted another 18 days.

In the majority of positive seedings of blood in the dishes individual colonies of pneumococci grew. In a number of mice the finding of the causative agent in the blood was repeated. However, the majority of animals from whose blood pneumococci were isolated remained alive for a prolonged period after the cessation of penicillin therapy (see table).

Thus the entering of a small number of pneumococci into the blood stream and their residence in it far from always led to the death of even such a sensitive animal as the mouse. A similar bacteremia may be temporary and therefore remain unexposed during subsequent seedings of blood. It is possible that for the development of a lethal sepsis in mice it is necessary to have present a considerable primary microbial focus. Analogous assumptions may be found in works devoted to the development of staphylococcus infection (Krantsfeld, 1896; Christovich, 1961, Grawitz and deBary, 1886; Koenig et al., 1962, and others).

Subsequently we attempted to follow bacteremia in mice which had been infected in the nose. The animals of one group were infected with a whole culture (4,400,000 pneumococci per mouse), and the other group -- with a culture diluted to 1:100 (correspondingly 44,000 microbes). Daily for the course of 7 days seedings from blood were made from each of the 10 mice in a group. In all cases when pneumococci were detected in seedings of blood, bacteremia was substantial and the animals died soon. In the first group of such mice there were two, and in the second group -- one. The remaining animals did not have perceptible amounts of microbes in their blood and remained alive.

The problem arose, did transient or space bacteremia, which was noted following infection of mice in the extremities with moderate or small doses of causative agent, actually terminate for the animals without leaving any trace. A similar question is also in order for the tests with the intranasal infection of mice, after which pneumococci were generally not detected in the blood of surviving animals. In order to answer this problem we studied the resistance to subsequent infection in mice which had survived following

infection in the extremities and in the nose. In series I of the tests the animals were infected in an extremity with 2 doses of a pneumococcus culture - 220 and 22 cells per mouse. After 11 days the surviving mice (a little more than 50% in each group), along with the control, were infected intraperitoneally with a 24-hour broth culture of pneumococci and the LD₅₀ was determined for each group. The mice which were preliminarily infected with a large dose of microbes turned out to be considerably more resistant than the control -- they survived following infection with 150,000 pneumococci. Even the mice which had preliminarily received in the extremity little more than 20 pneumococci survived from doses which equaled 702 microbes, that is, from a dose which exceeded by 78 times the dose from which the control animals survived (9 microbial cells).

Similar data were obtained also during the repeated infection of mice which had survived following infection in the extremity with a ten-fold increase in the dose of pneumococci. The greater the initial dose of infection, the more resistant the mice turned out to be to the repeated administration of pneumococci.

In series II of the experiments the mice received live or heat killed (30 minutes at 60°) pneumococci in the nose. In the course of 10 days, out of 100 mice infected with the live culture 10 died. For the surviving mice the magnitude of the LD₅₀ was titrated during intraperitoneal infection. For the control mice it equaled 3--4 cells, for the animals which preliminarily received the killed culture -- 239 cells, and for the animals preliminarily infected in the nose with the live culture -- 13,800 microbes.

In both series of tests along with the determination of the resistance of the surviving mice to subsequent intraperitoneal infection we studied the presence of agglutinins in their blood. For this the sera in various dilutions (beginning from 1:1.5 -- 1:5) were investigated in the agglutination reaction with a 24-hour broth culture of pneumococci. The reaction was negative in all cases.

The results of the tests indicate that not only a transient process, manifesting itself in a temporary scanty bacteremia, but even a symptomless purification of an organism in the event of a substantial infection through respiratory routes leaves behind itself a profound trace in the form of immunological shifts, showing up in an increase of resistance of the organism to subsequent infection by pneumococcus. At the same time the nasal administration to mice of a similar quantity of killed pneumococcus culture caused only a comparatively weak resistance in the animals. On the basis of these data it is possible to arrive at two assumptions: Either for an immunological response it is necessary that the organism endure similar mitigated infections, or following heating of the pneumococcus culture an attenuation of the original protective antigen sets in, and it is necessary to search for other methods of rendering the pneumococci harmless without destroying this antigen.

Conclusions

1. Following the infection of mice with pneumococci the following gradation in the decrease of sensitivity of the animals was established depending on the route of introduction of the microbes: Intraperitoneal injection, infection in the extremity, in the vein, and by the intranasal route.
2. Under specific conditions (infection with small doses in the extremity or infection following the use of moderate penicillin therapy) it is possible to observe a rapidly passing and weakly expressed bacteremia. The animals in which bacteremia was detected remained alive in a great number of cases.
3. The temporary and scanty bacteremia following infection of mice in the extremity, and also the symptomless process following the intranasal infection of animals, caused a profound immunological reorganization in them, as a result of which the mice became highly resistant to pneumococcus infection. This immunological response was not accompanied by the formation of specific agglutinins.

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Bacteremia and survival rate in mice, treated with penicillin,
following infection with type II pneumococcus

Index	Result of infection with a various amount of microbial cells		
	1050	105	10
Number of mice in which pneumococcus bacteremia was detected (one or more times)	11	8	10
Number of mice which died from the total number infected	6/14	2/12	3/16